PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12N 15/40, 15/82, 5/14
A01H 4/00, A01N 63/00

A1

(11) International Publication Number: WO 90/02184

(43) International Publication Date: 8 March 1990 (08.03.90)

(21) International Application Number: PCT/US89/03094

(22) International Filing Date: 20 July 1989 (20.07.89)

(30) Priority data:

234,412	19 August 1988 (19.08.88)	US
323,536	14 March 1989 (14.03.89)	US
368,710	19 June 1989 (19.06.89)	US

(60) Parent Application or Grant
(63) Related by Continuation
US 368,710 (CON)
Filed on 19 June 1989 (19.06.89)

(71) Applicants *(for all designated States except US):* THE UPJOHN COMPANY [US/US]; 301 Henrietta Street, Kalamazoo, MI 49001 (US). CORNELL RESEARCH FOUNDATION, INC. [US/US]; 20 Thornwood Drive, Ithaca, NY 14850 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only) :* SLIGHTOM, Jerry, L. [US/US]; 3305 Lorraine Avenue, Kalamazoo, MI 49007 (US). QUEMADA, Hector, D. [US/US]; 2524 E.G. Avenue, Kalamazoo, MI 49004 (US). GONSALVES, Dennis [US/US]; 595 Castle Street, Geneva, NY 14456 (US). L'HOSTIS, Brigitte [FR/US]; 607 Castle Street, Geneva, NY 14456 (US).

(74) Agent: DELUCA, Mark; Patent Law Department, The Upjohn Company, Kalamazoo, MI 49001 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent), SU, US.

Published
With international search report.

(54) Title: POTYVIRUS COAT PROTEIN GENES AND PLANTS TRANSFORMED THEREWITH

(57) Abstract

The present invention relates to the coat protein genes of Papaya Ringspot Virus Strain papaya ringspot (PRV-p), Watermelon Mosaic Virus II (WMVII), and Zucchini Yellow Mosaic Virus (ZYMV); to expression vectors which contain a coat protein gene for PRV-p, WMVII or ZYMV, and, additionally, the necessary genetic regulatory sequences needed for expression of a gene transferred into a plant; to bacterial or plant cells which are transformed with an expression vector containing the PRV-p, WMVII or ZYMV coat protein genes; to transgenic plants which are produced from plant cells transformed with an expression vector containing the coat protein gene from PRV-p, WMVII or ZYMV; and to a process of producing transgenic plants which have increased resistance to viral infection.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MR	Mauritania
BE	Belgium	GA	Gabon	MW	Malawi
BF	Burkina Fasso	GB	United Kingdom	NL	Netherlands
BG	Bulgaria	HU	Hungary	NO	Norway
BJ	Benin	IT	Italy	RO	Romania
BR	Brazil	JP	Japan	SD	Sudan
CA	Canada	KP	Democratic People's Republic of Korea	SE	Sweden
CF	Central African Republic			SN	Senegal
CG	Congo	KR	Republic of Korea	SU	Soviet Union
CH	Switzerland	LI	Liechtenstein	TD	Chad
CM	Cameroon	LK	Sri Lanka	TG	Togo
DE	Germany, Federal Republic of	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		

POTYVIRUS COAT PROTEIN GENES AND PLANTS TRANSFORMED THEREWITH

FIELD OF INVENTION

The present invention relates to the coat protein genes of potyviruses. More specifically the invention relates to a process for preparing a coat protein gene from a potyvirus as well as its incorporation into a transfer vector, and its use in producing transformed plant cells and transformed plants which are resistant to viral infections by the particular potyvirus and related viruses from which the gene is derived.

BACKGROUND OF THE INVENTION

Potyviruses are a distinct group of plant viruses which are pathogenic to various crops. Potyviruses include watermelon mosaic virus II (WMVII); papaya ringspot virus strains papaya ringspot and watermelon mosaic I (PRV-p and PRV-w), two closely related members of the plant potyvirus group which were at one time classified as distinct virus types, but are presently classified as different strains of the same virus; zucchini yellow mosaic virus (ZYMV); and many others. These viruses consist of flexous, filamentous particles of dimensions approximately 780 X 12 nanometers. The viral particles contain a single-stranded RNA genome containing about 10,000 nucleotides of positive (+, coding, or sense) polarity. Translation of the RNA genome of potyviruses shows that the RNA encodes a single large polyprotein of about 330 kD. This polyprotein contains several proteins, one of which is a 49kD protease that is specific for the cleavage of the polyprotein into at least six (6) other peptides. One of the proteins contained within this polyprotein is a 35kD capsid or coat protein which coats and protects the viral RNA from degradation.

The genome organization of several viruses belonging to the potyvirus family group has been studied in detail, in particular tobacco etch virus, tobacco vein mottling virus and pepper mottle virus. In each case, the location of the coat protein gene has been at the 3'-end of the RNA, just prior to a stretch of (200 to 300 bases) terminal adenine nucleotides residues. The location of the 49 kD protease gene appears to be conserved in these viruses. In the tobacco etch virus, the protease cleavage site has been determined to be the dipeptide Gln-Ser, Gln-Gly or Gln-Ala. Conservation of these dipeptides as the cleavage sites in these viral polyproteins is

apparent from the sequences of the above-listed potyviruses.

Expression of the coat protein genes from tobacco mosaic virus, alfalfa mosaic virus, cucumber mosaic virus, and potato virus X in transgenic plants has resulted in plants which are resistant to infection by the respective virus. In order to produce such transgenic plants, the coat protein gene must be inserted into the genome of the plant. Furthermore, the coat protein gene must contain all the genetic control sequences necessary for the expression of the gene after it has been incorporated into the plant genome.

Since the coat protein of a potyvirus is produced by the post translational processing of a polyprotein, the coat protein gene isolated from viral RNA does not contain the genetic regulatory sequences needed for gene expression. The coat protein gene does not contain the transcription and translation signals necessary for its expression once transferred and integrated into a plant genome. It must, therefore, be engineered to contain a plant expressible promoter, a translation initiation codon (ATG) and a plant functional poly(A) addition signal (AATAAA) 3′ of its translation termination codon.

In the present invention, the nucleotide sequences of the coat protein genes for WMV-II, PRV-p and ZYMV have been determined, and the genes have been inserted into expression vectors to supply them with the necessary genetic regulatory sequences so that the genes can be expressed when incorporated into a plant genome. Plant cells are transformed with the vector construct and the plant cells are induced to regenerate. The resulting plants contain the coat protein genes and produce the coat protein. The production of the protein confers upon the plant an increased resistance to infection by the virus from which the coat protein gene was derived.

INFORMATION DISCLOSURE

European patent application EP 0 223 452 describes plants that are resistant to viral diseases and methods for producing them. The process described comprises the steps of transforming a plant with a DNA insert comprising a promoter, a DNA sequence derived from the virus, and a poly(A) addition sequence.

PCT patent application PCT/US86/00514 refers generally to a method of conferring resistance to a parasite to a host of the parasite.

Allison et al. (1985) "Biochemical Analysis of the Capsid Protein Gene and Capsid Protein of Tobacco Etch Virus: N-Terminal Amino Acids Are Located on the Virion's Surface", Virology 147:309-316, describe the nucleotide sequence at the 3' end of the tobacco etch virus genome encoding the capsid protein. Homology to the sequence encoding the capsid protein of Pepper mottle virus is reported.

Allison et al. (1986) "The Nucleotide Sequence of the Coding Region of Tobacco Etch Virus Genomic RNA: Evidence for the Synthesis of a Single Polyprotein", Virology 154:9-20 describe the genome organization of the tobacco etch virus.

Carrington, J.C. and Dougherty, W.G. (1987) "Small nuclear inclusion protein encoded by a plant potyvirus genome is a protease", J. Virology 61:2540-2548, disclose that the viral RNA of tobacco etch virus encodes the 49K protease responsible for cleavage of the polyprotein produced when the viral RNA is translated.

Dodds et al. (1985) "Cross Protection between strains of cucumber mosaic virus: effect of host and type of inoculum on accumulation of virions and double-stranded RNA of the challenge strain", Virology 144:301-309, describe increased resistance to challenge by virus conferred to a plant by infection of a different strain of virus.

Dougherty, W.G. et al. (1985) "Nucleotide Sequence at the 3' Terminus of Pepper Mottle Virus Genomic RNA: Evidence for an Alternative Mode of Potyvirus Capsid Protein Gene Organization", Virology 146:282-291, report the nucleotide sequence of the 3' terminus of the viral RNA genome of pepper mottle virus.

Dougherty, W.G. et al. (1988) "Biochemical and mutational analysis of plant virus polyprotein cleavage site", EMBO J. 7:1281-1287, describe the conservation of the proteolytic cleavage site among geographically distinct isolates of tobacco etch virus.

Dougherty, W. G. and Carrington, J. C. (1988) "Expression and function of potyviral gene products", Ann. Rev. Phytopathol. 26:123-143, describe potyviruses and some of the similarities the members of the group have with each another.

Eggenberger, A. L. et al. (1989) "The nucleotide sequence of a Soybean Mosaic Virus Coat Protein region and its expression in Escherichia coli, Agrobacterium tumefaciens, and tobacco callus",

Virology, in press, disclose the nucleotide sequence of the coat protein gene for soy bean mosaic virus.

Hinchee, M. A. W. et al (1988) "Production of transgenic soybean plants using Agrobacterium-mediated DNA transfer", Bio/tech. 6:915-921, disclose the production of transgenic soybean plants which were transformed with A. tumerfacien plasmids that conferred either Kanamycin resistance/β-glucuronidase activity or Kanamycin resistance/glyphosphate tolerance.

Kozak, M. (1986) "Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes", Cell 44:283-292, discloses the optimal sequence around the ATG initiator codon of the preproinsulin gene for initiation by eukaryotic ribosomes.

Loesch-Fries et al. (1987) "Expression of alfalfa mosaic virus RNA 4 in transgenic plants confers virus resistance", EMBO J 6:1845-1851, disclose that expression of the coat protein gene of alfalfa mosaic virus in transgenic plants confers resistance to infection by the virus.

Pietrzak et al. (1986) "Expression in plants of two bacterial antibiotic resistant genes after protoplast transformation with a new plant expression vector", Nucleic Acids Research 14:5857-5868, disclose expression in plants of foreign genes introduced into the plant using an expression vector containing a movable expression cassette consisting of the Cauliflower mosaic virus 35S promoter and transcription terminator seperated by a polylinker containing several unique restriction sites.

Powell-Abel et al. (1986) "Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene", Science 232:738-743, disclose increased resistance toward infection by tobacco mosaic virus in transgenic plants containing the coat protein gene from tobacco mosaic virus.

Quemada, H. D. et al. (1989) "The nucleotide sequences of cDNA clones from RNA3 of Cucumber Mosaic Virus strains C and WL", J. Gen. Virol. 70:1065-1073, reports the nucleotide sequences of cDNA clones from RNA3 of Cucumber Mosaic Virus strains C and WL and compares them to each other and other strains for homology.

Shukla, D. D. et al. (1986) "Coat Proteins of Potyviruses", Virology 152:118-125, discloses the amino acid sequence of the potato

virus Y coat protein.

Shukla, D. D. et al. (1988) "The N and C termini of the Coat Proteins of Potyviruses Are Surface-located and the N Terminus Contains the Major Virus-specific Epitopes", J. Gen. Virol. 69:1497-1508, disclose that the N- and C-termini regions of some potyvirus coat proteins are located at the surface of the viral particles. The viral particles were treated with trypsin and it was observed that the enzyme treatment removed 30-67 amino acids from the N-terminal and 18-20 amino acids from the C-terminal; the variations were dependent on the virus. The remaining portion of the coat protein, the core, was highly conserved among the various viruses.

Tumer et al. (1987) "Expression of alfalfa mosaic virus coat protein gene confers cross-protection in transgenic tobacco and tomato plants", EMBO J. 6:1181-1188, disclose transgenic tobacco and tomato plants transformed with the coat protein gene of alfalfa mosaic virus display increased resistance to infection by alfalfa mosaic virus.

Yeh and Gonsalves (1985) "Translation of Papaya Ringspot Virus RNA in vitro: Detection of a Possible Polyprotein That is Processed for Capsid Protein, Cylindrical-Inclusion Protein, and Amorphous-Inclusion Protein", Virology 143:260-271, describe the possibility that the RNA genome encodes a single proprotein which undergoes post-translational processing to form the potyvirus protein products.

The following scientific publications are of interest but not relevant:

An et al. (1985) "New cloning vehicles for transformation of higher plants", EMBO J. 4:277-285 describe the construction of an expression plasmid which may be stably replicated in both E. coli and A. tumerfaciens.

An, G. (1986) "Development of plant promoter expression vectors and their use for analysis of differential activity of nopaline synthase promoter in transformed tobacco cells", Plant Physiol. 81:86-91, reports differences in promoter activities of transferred genes within the same cells as well as in independently derived cell lines.

Bevan et al. (1983) "Structure and transcription of the nopaline synthase gene region of T-DNA", Nucleic Acids Research 11:369-385,

disclose the DNA sequence and plant-tumor transcription pattern of a portion of DNA from A. tumerfaciens strain T37.

Depicker et al. (1982) "Nopaline synthase: transcript mapping and DNA sequence", J. Mol. Appl. Genet. 1:561-573, disclose the DNA sequences 5′ and 3′ to the nos gene found in A. tumerfaciens.

Hepburn, A. et al. (1985) "The use of pNJ5000 as an intermediate vector for genetic manipulation of Agrobacterium Ti-plasmids", J. General Microbio. 131:2961-2969, describe vectors which are used to transfer narrow host range vectors from E. coli to A. tumerfaciens.

Klein et al., (1987) "High-velocity microprojectiles for delivering mucleic acids into living cells", Nature 327:70-73, disclose that nucleic acids may be delivered into living cells using accelerated, small tungsten balls which pierce the cells without killing them.

Klein et al., (1988) "Factors influencing gene delivery into Zea mays cells by high-velocity microprojectiles", Bio/tech. 6:559-563, disclose that two days after bombarding plant cells with a plasmid coated microprojectile, expression of an gene encoding an enzyme could be detected.

Mazur, B. J. and Chui, C.-F. (1985) "Sequence of a genomic DNA clone for the small subunit of ribulose bis-phosphate carboxylase-oxygenase from tobacco", Nucleic Acids Research 13:2373-2386, disclose the DNA sequence of the small subunit of ribulose bis-phosphate carboxylase-oxygenase from tobacco.

McCabe, D. E., et al., (1988) "Stable transformation of soybean (Glycine max) by particle acceleration", Bio/tech. 6:923-926, disclose expression in soybean shoots of foreign genes introduced into immature soybean seeds using DNA coated microprojectiles.

Olson, M. K. et al (1989) "Enhancement of heterologous polypeptide expression by alterations in the ribosome-binding-site sequence", J. Biotech. 9:179-190, discloses the increase in gene expression of heterologous genes in E. coli due to the presence of an AT-rich 5′ untranslated region.

Slightom et al. (1983) "Complete nucleotide sequence of a French bean storage protein gene: Phaseolin", Proc. Natl. Acad. Sci. U.S.A. 80:1897-1901, disclose the complete nucleotide sequences of the gene and the mRNA coding for a specific phaselin type French bean major storage protein.

Vilaine, F. and Casse-Delbart, F. (1987) "Independent induction of transformed roots by the TL and TR regions of the Ri plasmid of agropine type Agrobacterium rhizogenes", Mol. Gen. Genet. 206:17-23, disclose the respective role of Tl- and TR-DNA in root induction by agropine type Agrobacterium rhizogenes Ri plasmids.

None of these documents, either alone or taken together, teaches or suggests the instant invention which relates to potyvirus coat protein genes and plants transformed therewith.

SUMMARY OF THE INVENTION

The present invention relates to the coat protein genes of Papaya Ringspot Virus Strain papaya ringspot (PRV-p), Watermelon Mosaic Virus II (WMVII), and Zucchini Yellow Mosaic Virus (ZYMV).

The present invention relates to a recombinant DNA molecule which encodes a potyvirus coat protein. The present invention relates to a recombinant DNA molecule comprising a potyvirus coat protein gene operably linked to genetic regulatory sequences necessary for gene expression.

The present invention relates to expression vectors which contain a coat protein gene for potyviruses, and, additionally, the necessary genetic regulatory sequences needed for expression of a gene transferred into a plant. The present invention also relates to bacterial or plant cells which are transformed with an expression vector containing the coat protein genes. Furthermore, the present invention relates to transgenic plants which are produced from plant cells transformed with an expression vector containing the coat protein gene from potyviruses. In addition, the present invention relates to a process of producing transgenic plants which have increased resistance to viral infection.

DETAILED DESCRIPTION OF THE INVENTION

Charts 1, 2 and 3 contain DNA nucleotide sequences of the coat protein genes of PRV-p, WMVII and ZYMV, respectively. Charts 4 and 5 compare the nucleotide sequences of various coat protein genes. Charts 6-14 are set forth to illustrate the constructions of this invention. Certain conventions are used to illustrate plasmids and DNA fragments as follows:

(1) The single line figures represent both circular and linear double-stranded DNA.

(2) Asterisks (*) indicate that the molecule represented is

circular. Lack of an asterisk indicates the molecule is linear.

(3) Junctions between natural boundaries of functional components are indicated by vertical lines along the horizontal lines.

(4) Genes or functional components are indicated below the horizontal lines.

(5) Restriction sites are indicated above the horizontal lines.

(6) Distances between genes and restriction sites are not to scale. The figures show the relative positions only unless indicated otherwise.

(7) The following abbreviations are used to denote function and components:

a) P_{ca} = CaMV35S promoter;

b) I_c = CMV intergenic region, the intergenic region comprising the initiation codon and AT rich 5' untranslated region;

c) S_{ca} = CaMV35S poly(A) addition signal; and

d) Nos = Nos nptII gene.

Most of the recombinant DNA methods employed in practicing the present invention are standard procedures, well known to those skilled in the art, and described in detail in, for example, European Patent Application Publication Number 223452, published November 29, 1986, which is incorporated herein by reference. Enzymes are obtained from commercial sources and are used according to the vendor's recommendations or other variations known in the art. General references containing such standard techniques include the following: R. Wu, ed. (1979) Methods in Enzymology, Vol. 68; J. H. Miller (1972) Experiments in Molecular Genetics; T. Maniatis et al. (1982) Molecular Cloning: A Laboratory Manual; D. M. Glover, ed. (1985) DNA Cloning Vol. II; H.G. Polites and K.R. Marotti (1987) "A step-wise protocol for cDNA synthesis". Biotechniques 4:514-520; S.B. Gelvin and R.A. Schilperoort, eds. Introduction, Expression, and Analysis of Gene Products in Plants, all of which are incorporated by reference.

For the purposes of the present disclosure the following definitions apply.

"Promoter" means a promoter which is functional in the host

plant.

"Initiation region" includes the initiation codon and nucleotides flanking the initiation codon.

"Operably linked" refers to the linking of nucleotide regions encoding specific genetic information such that the nucleotide regions are contiguous, the functionality of the region is preserved and will perform its function relative the the other regions as part of a functional unit.

"AT rich 5' untranslated region" is a nucleotide sequence composed of at least 60% adenine or thymine nucleotides.

"Untranslated flanking region" refers to nucleotide sequences which are 3' of the termination codon and end at the poly(A) addition signal. These sequences enhance production of the peptide encoded by the upstream gene.

"Vector" is a vehicle by means of which DNA fragments can be introduced into host organisms.

"Expression vector" is a vehicle by means of which DNA fragments that contain sufficient genetic information and can, therefore, be expressed by the host, can be introduced into host organisms.

"Antipathogen gene" is a gene which encodes a DNA sequence which is either the antisense sequence of a pathogenic gene or the antipathogenic gene encodes a peptide whose presence in an organism confers an increased resistence to a pathogen.

To practice the present invention, the coat protein gene of a virus must be isolated from the viral genome and inserted into a vector containing the genetic regulatory sequences necessary to express the inserted gene. Accordingly, a vector must be constructed to provide the regulatory sequences such that they will be functional upon inserting a desired gene. When the expression vector/insert construct is assembled, it is used to transform plant cells which are then used to regenerate plants. These transgenic plants carry the viral gene in the expression vector/insert construct. The gene is expressed in the plant and increased resistance to viral infection is conferred thereby.

Several different courses exist to isolate the coat protein gene. To do so, one having ordinary skill in the art can use information about the genome organization of potyviruses to locate and isolate the coat protein gene. The coat protein gene is located

at the 3' end of the RNA, just prior to a stretch of about 200-300 adenine nucleotide residues. Additionally, the information related to proteolytic cleavage sites is used to determine the N-terminus of the potyvirus coat protein gene. The protease recognition sites are conserved in the potyviruses and have been determined to be either the dipeptide Gln-Ser, Gln-Gly or Gln-Ala. The nucleotide sequences which encode these dipeptides can be determined.

Using methods well known in the art, a quantity of virus is grown up and harvested. The viral RNA is then seperated and the coat protein gene can be isolated using a number of known procedures. A cDNA library is created using the viral RNA. The methods followed to do this are well known in the art. The viral RNA is treated with reverse transcriptase and a complementary DNA molecule is produced. A DNA complement of the complementary DNA molecule is produced and that sequence represents a DNA copy of the original viral RNA molecule. Thus, a double stranded DNA molecule is generated which contains the sequence information of the viral RNA. These DNA molecules can be cloned in E. coli plasmid vectors after the additions of restriction enzyme linker molecules by DNA ligase. The various fragments are inserted into cloning vectors which are then used to transform E. coli and create a cDNA library.

Since the coat protein gene is located just 5' to the polyA region, oligonucleotides that can hybridize to the polyA region can be used as hybridization probes to screen the cDNA library and determine if any of the transformed bacteria contain DNA fragments with sequences coding for the polyA region. The cDNA inserts in any bacterial colonies which contain this region can be sequenced. The coat protein gene is present in its entirety in colonies which have sequences that extend 5' to the sequence which encodes the proteolytic cleavage site described above.

Alternatively, cDNA fragments may be inserted into expression vectors. Antibodies against the coat protein may be used to screen the cDNA expression library and the gene can be isolated from colonies which express the protein.

Using the sequences disclosed in Charts 1, 2 and 3, the coat protein genes for the respective viruses may be synthesized chemically by methods well known in the art. Alternatively, the information in Charts 1, 2 and 3 may be used to synthesize

oligonucleotides which can be used as probes to .screen a cDNA library.

The nucleotide sequences of the coat protein genes for WMV-II, PRV-p and ZYMV have been determined and the genes have been inserted into expression vectors. The expression vectors contain the necessary genetic regulatory sequences for expression of an inserted gene. The coat protein gene is inserted such that those regulatory sequences are functional so that the genes can be expressed when incorporated into a plant genome.

In order to express the viral gene, the necessary genetic regulatory sequences must be provided. Since the coat protein of a potyvirus is produced by the post translational processing of a polyprotein, the coat protein gene isolated from viral RNA does not contain the genetic regulatory sequences needed for gene expression. The coat protein gene does not contain the transcription and translation signals necessary for its expression once transferred and integrated into a plant genome. It must, therefore, be engineered to contain a plant expressible promoter, a translation initiation codon (ATG) and a plant functional poly(A) addition signal (AATAAA) 3' of its translation termination codon. In the present invention, the coat protein is inserted into a vector which contains a cloning site for insertion 3' of the initiation codon and 5' of the poly(A) signal. The promoter is 5' of the initiation codon such that when a structural gene is inserted at the cloning site, a functional unit is formed in which the inserted gene is expressed under the control of the various genetic regulatory sequences.

In the preferred embodiment of the present invention, additional genetic regulatory sequences are provided. As described above, an expression vector must contain a promoter, an intiation codon and a poly(A) addition signal. In order to get a higher level of expression, untranslated regions 5' and 3' to the inserted genes are provided. Furthermore, certain sequences flanking the initiation codon optimize expression. The promoter used is one that is chosen for high level expression.

A 5' untranslated region which results in higher level expression of an inserted gene is provided downstream from the promoter and upstream from the initiation codon. This region contains at least 60% of the sequence a Adenine and Thymine. There is a statistical

bias for expression when such an AT rich region is located between the promoter and intiation codon. This preference is utilized in the preferred embodiment of the present invention by inclusion of an AT rich 5' untranslated region intermediate of the promoter and initiation codon.

The preferred embodiment of the present invention also contains specific nucleotide sequence flanking the initiation codon. This preferred sequence, termed Kozak's element, is AAXXATGG wherein X represents any of the four nucleotides. The presence of the initiation codon following Kozak's rule results in higher level expression when used in an expression vector. In the preferred embodiment of the present invention, the small subunit from the SS RUBISCO contains an initiation codon in which Kozak's element is used.

Furthermore, the preferred embodiment of the present invention contains a 3' untranslated region downstream from the cloning site where the coat protein gene is inserted and upstream from the poly(A) addition signal. The sequence of this 3' untranslated region results in a statistical bias for protein production. The sequence promotes high level expression. The poly(A) addition signal is found directly downstream from the 3' untranslated region and can be derived from the same source. In the preferred embodiment of the present invention, the 3' untranslated region and poly(A) addition signal are derived from CaMV 35S gene or the phaseolin seed storage protein gene.

The poly(A) addition signal from CaMV, nopaline synthase, octopine synthase, bean storage protein, and SS RUBISCO genes are also suitable for this construction. Several promoters which function in plants are available, but the best promoters are the constitutive promoter from cauliflower mosaic virus (CaMV, a plant DNA virus) and the small subunit of ribulose bis-phosphate carboxylase-oxygenase (SS RUBISCO) gene.

Using methods well known to those skilled in the art, plant cells are transformed with the vector construct and the plant cells are induced to regenerate. The resulting plants contain the coat protein genes and produce the coat protein. The production of the protein confers upon the plant an increased resistance to infection by the virus from which the coat protein gene was derived.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Example 1 Isolation of WMVII RNA

Watermelon mosaic virus II (WMV II) was propagated in zucchini squash (Cucurbita pepo L) plants and RNA was isolated by the method described by Yeh and Gonsalves (Virology 143:260, 1985).

Example 2 Isolation of PRV-p RNA

Papaya ringspot virus strain prv (PRV-p) was propagated in jelly melon, Cucumis metuliferus (Nand.) Mey. Acc. 2549 plants and RNA was isolated by the method described by Yeh and Gonsalves (Virology 143:260, 1985).

Example 3 Isolation of ZYMV RNA

Zucchini yellow mosaic virus (ZYMV) was propagated in zucchini squash (Cucurbita pepo L) plants and RNA was isolated by the method described by Yeh and Gonsalves (Virology 143:260, 1985).

Example 4 Synthesis of double-stranded cDNA

The procedure used to make double stranded cDNA from isolated viral RNA is the same for all viral RNA isolated above. The purified RNA was subjected to the cDNA synthesis protocol described by Polites and Marotti (Biotechniques 4:514, 1986) and because this RNA contains an A-rich region at its 3'-end (similar to that found for many eukaryotic mRNAs) the procedure was straight-forward. The synthesis of double stranded cDNA was also done as described by Polites and Marotti. After double-stranded cDNA was synthesized, it was purified by passage through a G-100 Sephadex column, precipitated with ethanol, and suspended in 20 μl of 10X EcoRI methylase buffer (100 mM NaCl, 100 mM Tris-HCl, pH 8.0, 1 mM EDTA, 80 μM S-adenosyl methionine, and 100 μg/ml bovine serum albumin). An additional amount of S-adenosyl methionine (1 μl of a 32 mM solution) was added to the reaction mixture, followed by the addition of 1 μl (20 units) EcoRI methylase. The reaction was incubated at 37°C for 30 minutes and stopped by incubation at 70°C for 10 minutes. Then 1 μl (5 units) of E. coli DNA polymerase I Klenow fragment was added and incubated at 37°C for 10 minutes, followed by phenol/chloroform extraction and ethanol precipitation. The pellet was washed in 70% ethanol, then 70% ethanol/0.3 M sodium acetate. The pellet was dried and resuspended in 8 μl of 0.5 μg/μl phosphorylated EcoRI linkers (Collaborative Research, Inc., 128 Spring St., Lexington, MA 02173). One μl of 10X ligase buffer (800 mM Tris-HCl ph 8.0, 200 mM $MgCl_2$.

150 mM DTT, 10 mM ATP) and 1 μl of T4 DNA ligase (4 units) were added, and the reaction was incubated overnight at 15°C. The ligation reaction was stopped by incubation at 65°C for 10 minutes. Sixty μl of H_2O, 10 μl of 10X EcoRI salts (900 mM Tris-HCl pH 8.0, 100 mM $MgCl_2$, 100 mM NaCl), and 10 μl of EcoRI (10 units/μl) were added, and the reaction was incubated at 37°C for 1 hour. The reaction was stopped by phenol/chloroform and chloroform extractions. The reaction mixture was then size fractionated by passage through a Sephadex G-100 column and the fractions containing the largest double stranded cDNA molecules were concentrated by butanol extractions, precipitated with ethanol, and resuspended in 10 μl of H_2O. Five μl of the double stranded cDNAs was added to 0.5 μg of pUC19 DNA (which had been previously treated with phosphatase to remove the 5' phosphates), 1 μl of 10X ligase buffer, and 1 μl of T4 ligase, and the reaction was incubated at 15°C for 16 hours. The resulting ligated pUC19-coat protein gene double stranded cDNA molecules were transformed into E.coli host cells as described by the manufacturer (Bethesda Research Laboratories, Inc., Gaithersburg, MD 20877) and plated on medium containing 50 μg/ml ampicillin, 0.04 mM IPTG, and 0.004% X-Gal. Bacterial colonies showing no blue color were selected for further analysis. Clones containing the 3'-region and possibly the coat protein gene were identified by hybridization against a ^{32}P-labeled oligo-dT. Bacterial colonies showing hybridization to this probe should contain at least the poly(A) region of the particular potyvirus genome. Several of the hybridizing bacterial clones were selected and plasmid DNAs were isolated according to methods known to those skilled in the art.

Example 5 Identification of the PRV-p Coat Protein Gene

Several of the cloned cDNAs of PVP-p RNA were sequenced by the chemical DNA sequencing method described by Maxam and Gilbert (Methods of Enzymology 65:499, 1980). Based on this information and comparative analysis with other potyviruses clone number pPRV-117 was found to contain a complete copy of the PRV-p coat protein gene. The N-terminus of the coat protein was identified by the location of the dipeptide sequence Gln-Ser. The length of the PRV-p coat protein gene coding region is consistent with a gene encoding a protein of about 33 kDal. The sequence of the PRV-p coat protein gene and protein are shown in Chart 1. In addition, comparison of this

sequence with that of the related virus PRV-w described by Nagel and Heibert (Virology 143:435, 1985) shows that the two coat protein genes share 98% nucleotide and amino acid similarities (Chart 4). Because these two viruses share extensive identities in their coat proteins, expression of the coat protein gene from PRV-p is expected to yield plants resistant to both PRV-p and PRV-w.

Example 6 . Construction of a Plant-expressible PRV-p Coat Protein Gene Cassette with CaMV 35S Promoter and Polyadenylation Signal and CMV 5' Untranslated Region and Translation Initiator ATG.

Attachment of the necessary plant regulatory signals to the PRV-p coat protein gene was accomplished by constructing a translational fusion with a clone originally designed for the expression of the CMV coat protein gene, using clone pUC1813/CP19. Plasmid pUC1813/CP19 is a vector suitable for agrobacterium mediated gene transfer. An EcoRI-EcoRI fragment was removed from pDH51/CP19 and placed into the EcoRI site of the plasmid, pUC1813 (available from Robert K., Department of Chemistry, Washington State University, Pullman, Washington), creating plasmid pUC1813/CP19. Plasmid pUC1813/CP19 was described in U.S. patent application Serial Number 07/135,591 filed on December 21, 1987 incorporated herein by reference. This translational fusion clone was constructed by first identifying two restriction enzyme sites within clone pUC1813/CP19. One site (Tth111 I) is located between amino acids 13 to 17 while the other site (BstX I) is located near the end of the 3'-untranslated region of the CMV coat protein gene.

Addition of these specific restriction enzyme sites to the PRV-p coat protein gene was accomplished by the polymerase chain reaction technique, using an instrument and Taq polymerase purchased from Perkin Elmer-Cetus, Emeryville, Ca. Specifically, two respective 5' and 3' oligomers (CGACGTCGTCAGTCCAAGAATGAAGCTGTG, containing a Tth111 I site and (CCCACGAAAGTGGGGTGAAACAGGGTCGAGTCAG, containing a BstX I site), sharing at least 20 nucleotides with the PRV-p coat protein gene were used to prime synthesis and gene amplification of the coat protein gene. After synthesis, the amplified fragments were digested with Tth111 I and BstX I to expose the sites.

As shown in Chart 6, pUC1813/CP19 is the expression vector which contains the CMV coat protein gene. Plasmid pUC1813/CP19 contains Tth111 I and BstX I sites.

The digested, amplified fragments are ligated into the respective exposed sites of pUC1813/CP19 and the expected new construction was identified using methods known to those skilled in the art. Polymerase chain reaction techniques were used to amplify PRV-P coat protein gene containing the Tth111I and BstXI sites. The plasmid pUC1813/CP19 and PRV-P coat protein gene fragments were digested with Tth111I and BstXI and ligated to each other. The resulting clone, designated pUC1813/CP19-PRVexp, was subjected to nucleotide sequencing to ensure that the cloning and gene amplification did not introduce any detrimental artifacts. The sequence showed no artifacts, suggesting that this plant expression cassette should be capable of expressing a PRV-p coat protein gene which contains an additional 16 amino acids of CMV coat protein at its N-terminus.

Example 7 Construction of a Micro T-DNA Plasmid Containing the Plant-expressible PRV-p Coat Protein Gene Construction.

As depicted in Chart 7, the plant expression cassette for the PRV-p coat protein gene was transferred into a suitable micro T-DNA vector which contains the necessary Agrobacterium T-DNA transfer signals for transfer from an Agrobacterium and integration into a plant genome, and a wide host-range origin of replication (for replication in Agrobacterium). Plasmid pUC1813/CP19-PRVexp was digested with Hind III and the resulting 2.2 kb insert fragment containing the plant-expressible cassette was removed and ligated into the Hind III site of the modified Agrobacterium-derived micro-vector pGA482 (modification included the addition of the β-glucuronidase gene). The micro T-DNA vector, pGA482, is available from G. An, Institute of Biological Chemistry, Washington State University, Pullman, WA. The resulting plasmid was designated, pGA482/G/CP19-PRVexp and is shown in Chart 7. This plasmid (or derivatives thereof) was transferred into virulent or avirulent strains of Agrobacterium tumefaciens or rhizogenes, such as A208, C58, LBA4404, C58Z707, A4RS, A4RS(pRiB278b), and others. Strains A208 C58, LBA4404, and A4RS are available from American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD. Bacteria A4RS(pRiB278b)is available from Dr. F. Casse-Delbart, C.N.R.A., Routede Saint Cyr. F78000, Versailles, France. Strain C58Z707 is available from Dr. A.G.Hepburn, Dept. of Agronomy, University of Illinois, Urbana, IL.

After transfer of the engineered plasmid pGA482/G/CP19-PRVexp into any of the above listed Agrobacterium strains, these Agrobacterium strains can be used to transfer and integrate within a plant genome the plant-expressible PRV-p coat protein gene contained within its T-DNA region. This transfer can be accomplished using the standard methods for T-DNA transfers which are known to those skilled in the art, or this transfer can be accomplished using the methods described in a U.S. patent application Serial Number 07/135,655, filed on December 21, 1987, entitled "Agrobacterium Mediated Transformation of Germinating Plant Seeds" and incorporated herein by reference.

Example 8 Construction of a Plant-expression Cassette for Expression of Various Genes in Transgenic Plants.

In the preferred embodiment of the present invention, the following expression cassette was constructed to provide the necessary plant regulatory signals (which include the addition of a promoter, 5' untranslated region, translation initiation codon, and polyadenylation signal) to the gene inserts in order to achieve high level expression of the inserts. The expression cassette may be used to express any genes inserted therein. Accordingly, the applicability of the expression cassette goes beyond its use in expressing coat protein genes. Rather, the expression cassette may be used to express any desired protein in transgenic plants. The expression cassette is the preferred expression system for expressing viral coat protein genes in plants.

The expression cassette of the preferred embodiment contains: a constitutive promoter; a 5' untranslated region which enhances gene expression; an initiation codon which comprise Kozak's element; a cloning site where the gene to be expressed may be inserted to produce a functional expression unit; and a 3' untranslated region which comprises a poly(A) addition signal and untranslated flanking regions which result in a higher level of expression.

More specifically, the expression cassette which is the preferred embodiment of the present invention consists of the cauliflower mosaic virus (CaMV) 35S transcript promoter, the 5'-untranslated region of cucumber mosaic virus (CMV), the CMV translation initiation codon, and the CaMV polyadenylation signal. The construction of this expression cassette utilized the Polymerase

Chain Reaction (PCR) technique to obtain correct position of the plant regulatory signals and the addition of convenient restriction enzyme sites which allow for the easy addition of a coat protein gene and the excision of the completed cassette so it can be transferred to other plasmids.

To accomplish the construction of this expression cassette the following oligomers were synthesized:

1. 5'-GAAGCTTCCGGAAACCTCCTCGGATTCC-3', contains a HindIII site at its 5'-end and contains 21 bases which are identical to 21 bases in the 5'-flanking region of CaMV.

2. 5'-GCCATGGTTGACTCGACTCAATTCTACGAC-3', contains a NcoI site at its 5'-end which contains a translation initiation codon which conforms to Kozak's rules and has 21 bases which are identical to 21 bases in the antisense strand of the CMV 5'-untranslated region.

3. 5'-GCCATGGTTGCGCTGAAATCACCAGTCTC-3', contains a NcoI site at its 5'-end (which contains the same translation initiation codon as oligomer 2) and has 20 bases which are identical to 20 bases in the 3'-untranslated region of CaMV.

4. 5'-GAAGCTTGGTACCACTGGATTTTGGTT-3', contains a HindIII site at its 3'-end and has a 20 base match with the flanking DNA region 3' of the CaMV polyadenylation site (on the antisense strand).

These oligomers were used to amplify sequences contained within the CMV expression clone referred to as pUC1813/CP19, shown in Chart 6, and referred to above. As depicted in Chart 8, the PCR technique was used to amplify the gene regulatory regions in pUC1813/CP19. Amplification of the 5'-flanking, CMV 5'-untranslated region, and CMV initiation codon (which was modified to conform to Kozak's rule AAXXATGG) resulted in a fragment of about 400 base pairs in length and amplification of the CaMV 3-untranslated and flanking regions resulted in a fragment of about 200 base pairs in length. These fragments were digested with NcoI and HindIII, isolated from a polyacrylamide gel, and then ligated into HindIII digested and phosphatase treated pUC18. The resulting clone is referred to as p18CaMV/CMV-exp and is shown in Chart 8.

Example 9 Identification of the WMVII Coat Protein Gene

The cloned WMVII cDNA insert from clone pWMVII-41-3.2 which was produced as described above, was sequenced by using both the chemical (Maxam and Gilbert, Methods of Enzymology 65:499, 1980) and enzymatic

(Sanger et al., Proc. Natl. Acad. Sci. U.S.A. 74:5463, 1977) methods. Based on this information and comparative analysis with other potyviruses, the nucleotide sequence of clone pWMVII-41-3.2 was found to contain a complete copy of the WMVII coat protein gene. The N-terminus of the coat protein was suggested by the location of the dipeptide sequence Gln-Ser. The length of the WMVII coat protein gene coding region (281 amino acids) is consistent with a gene encoding a protein of about 33 kD. The sequences of this WMVII coat protein gene and protein are shown in Chart 2. In addition, comparison of this sequence with that obtained from the related virus Soybean Mosaic Virus (SMV) strain N described by Eggenberger et al. shows that they share overall about 88% identity and excluding the N-terminal length differences they share about 92.5% identity, see Chart 5. Because these two virus coat proteins share extensive amino acid identities, expression of the coat protein gene from WMVII is expected to yield plants resistant to WMVII infection and could yield plants resistant to SMV infection.

Example 10 Construction of a Plant-expressible WMVII Coat Protein Gene Cassette with CaMV 35S Promoter and Polyadenylation Signal and CMV Intergenic Region and Translation Initiator ATG.

As depicted in Chart 9, attachment of the necessary plant regulatory signals to the WMVII coat protein gene was accomplished by using the PCR technique to amplify the WMVII coat protein gene using oligomers which would add the necessary sites to its 5' and 3' sequences. Following this amplification the resulting fragment is digested with the appropriate restriction enzyme and cloned into the NcoI site of the above described expression cassette containing plasmid, p18CaMV/CMV-exp. Clones containing the WMVII coat protein gene insert need only be checked to determine correct orientation with respect with the CaMV promoter. However, to ensure that no artifacts have been incorporated during the PCR amplification the entire coat protein gene region is checked by nucleotide sequence analysis.

To obtain the amplified WMVII coat protein gene with NcoI restriction enzyme sites on both ends the following two oligomers were synthesized:

1. 5'-ACCATGGTGTCTTTACAATCAGGAAAAG-3', which adds a NcoI site to the 5'-end of the WMVII coat protein gene and retains the same ATG

translation start codon which is present in the expression cassette, p18CaMV/CMV-exp.

2. 5'-ACCATGGCGACCCGAAATGCTAACTGTG-3', which adds a NcoI site to the 3'-end of the WMVII coat protein gene, this site can be ligated into the expression cassette, p18CaMV/CMV-exp.

The cloning of this PCR WMVII coat protein gene, using these two oligomers, into p18CaMV/CMV-exp yields a plant expressible WMVII gene (referred to as p18WMVII-exp) which, following transcription and translation, will generate a WMVII coat protein which is identical to that derived from the WMVII coat protein gene nucleotide sequence, see Chart 2. However, this coat protein will differ, because of necessary genetic engineering to add the ATG initiation codon and by including the last four amino acids of the 54 kD nuclear inclusion protein (which is adjacent to the Glu-Ser protease cleavage site); the amino acids added are Val-Ser-Leu-Glu-N-ter WMVII. The addition of these four amino acid residues should not affect the ability of this coat protein to yield plants which are resistant to WMVII infections, because the N-terminal region of potyvirus coat proteins appear not to be well conserved for either length or amino acid identity. However, if this is found to be a problem its replacement would involve the use of a different oligomer to obtain N-terminal variations of the WMVII coat protein gene. The cloned construction of the plant expressible WMVII coat protein gene is referred to as p18WMVII-exp, and is shown in Chart 9.

Example 11 Construction of a Micro T-DNA Plasmid Containing the Plant-expressible WMVII Coat Protein Gene Construction.

As depicted in Chart 10, the plant expression cassette for the WMVII coat protein gene was transferred into a suitable micro-T-DNA vector which contains the necessary Agrobacterium T-DNA transfer signals (to mediated transfer from an Agrobacterium and integration into a plant genome) and wide-host range origin of replication (for replication in Agrobacterium) to form plasmid pGA482/G/CPWMVII-exp. To construct this plasmid, plasmid p18WMVII-exp was digested with Hind III (which cuts within the polycloning sites of pUC18, well outside of the expression cassette), and an 1.8 kb fragment containing the plant-expressible cassette was removed and ligated into the Hind III site of the modified Agrobacterium-derived micro-vector pGA482 (modification included the addition of the β-

glucuronidase gene). The micro T-DNA vector, pGA482, is shown in Chart 7 and available from G. An, Institute of Biological Chemistry, Washington State University, Pullman, WA. The resulting plasmid was designated, pGA482/G/CPWMVII-exp is shown in Chart 10. This plasmid (or derivatives thereof) was transferred into virulent or avirulent strains of Agrobacterium tumefaciens or rhizogenes, such as A208, C58, LBA4404, C58Z707, A4RS, A4RS(pRiB278b), and others. Strains A208 C58, LBA4404, and A4RS are available from American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD. Bacteria A4RS(pRiB278b)is available from Dr. F. Casse-Delbart, C.N.R.A., Routede Saint Cyr. F78000, Versailles, France. Bacteria C58Z707 is available from Dr. A.G.Hepburn, Dept. of Agronomy, University of Illinois, Urbana, IL.

After transfer of the engineered plasmid pGA482/G/CPWMVII-exp into any of the above listed Agrobacterium strains, these Agrobacterium strains can be used to transfer and integrate within a plant genome the plant-expressible WMVII coat protein gene contained within its T-DNA region. This transfer can be accomplished using the standard methods for T-DNA transfers which are known to those skilled in the art, or this transfer can be accomplished using the methods described in U.S. Patent application SN 07/135,655 filed December 21, 1987 entitled "Agrobacterium Mediated Transformation of Germinating Plant Seeds". In addition, it has recently been shown that such Agrobacteria are capable of transferring and integrating their T-DNA regions into the genome of soybean plants. Thus these strains could be used to transfer the plant expressible WMVII coat protein gene into the genome of soybean to develop a soybean plant line which is resistant to infection from soybean mosaic virus strains.

Example 12 Microprojectile Transfer of pWMVII-exp into Plant Tissues.

Recently an alternative approach for the transfer and integration of DNA into a plant genome has been developed. This technique relies on the use of microprojectiles on which the DNA (plasmid form) is attached. These microprojectiles are accelerated to high velocities and their momentum is used to penetrate plant cell walls and membranes. After penetration into a plant cell the attached DNA leaches off the microprojectile and is transferred to the nucleus where DNA repair enzymes integrate the "free" DNA into

the plant genome. In its present form the process is entirely random, but plant tissues which have been successfully transformed by the plasmid DNA (or part of it) can be identified and cultured to homogeneity by the use of selectable marker genes (such as the bacterial neomycin phosphotransferase II gene, NPTII), or reporter genes (such as the bacterial beta-glucuronidase gene, Gus). Successful use of particle acceleration to transform plants has recently been shown for soybean and the transfer of p18WMVII-exp into the genome could result in obtaining soybean plants which are resistant to infections from soybean mosaic virus strains.

The use of this process for the transfer of p18WMVII-exp can be accomplished after the addition of either plant expressible genes NPTII or Gus genes or both. Plasmids that have the nptII and Gus genes to p18WMVII-exp are shown in Chart 11, and referred to as p18GWMVII-exp and p18NGWMVII-exp. In addition, the construction described in Example 11 can also be used for microprojectile transfer as it already has both the nptII and Gus genes attached to the pWMVII-exp cassette (see Chart 10). The only difficulty which the use of pGA482GG/cpWMVII-exp may encounter during transfer by the microprojectile process is due to its large size, about 18kb, which may have a lower efficiency transfer and such larger plasmid generally yield less DNA during propagation.

To construct plasmid p18GWMVii-exp, plasmid p18WMVii-exp is digested with BamHI and ligated with a 3.0 kilobase BamHI isolated fragment containing the Gus gene. To construct plasmid p18NGWMVii-exp, the plasmid p18GWMVii-exp is digested with SmaI and ligated with a 2.4 kb isolated fragment containing the Nos-nptII gene generated by digestion with DraI and StuI.

Example 13 Identification of the ZYMV Coat Protein Gene.

The cloned ZYMV cDNA insert from clone pZYMV-15, which was cloned using the method described above, was sequenced by using both the chemical (Maxam and Gilbert, Methods of Enzymology 65:499, 1980) and enzymatic (Sanger et al., Proc. Natl. Acad. Sci. U.S.A. 74:5463, 1977) methods. Based on this information and comparative analysis with other potyviruses the nucleotide sequence of clone pZYMV-15 was found to contain a complete copy of the ZYMV coat protein gene. The N-terminus of the coat protein was suggested by the location of the dipeptide sequence Gln-Ser which is characteristic of cleavage sites

in potyviruses (see Dougherty et al. EMBO J. 7:1281, 1988). The length of the ZYMV coat protein gene coding region (280 amino acids) is consistent with a gene encoding a protein of about 31.3 kD. The sequences of this ZYMV coat protein gene and protein are shown in Chart 3.

Example 14 Construction of a Plant-expressible ZYMV Coat Protein Gene Cassette with CaMV 35S Promoter and Polyadenylation Signal and CMV Intergenic Region and Translation Initiator ATG.

As depicted in Chart 12, attachment of the necessary plant regulatory signals to the ZYMV coat protein gene was accomplished by using the PCR technique to amplify the ZYMV coat protein gene using oligomers which would add the necessary sites to its 5' and 3' sequences. Following this amplification the resulting fragment is digested with the appropriate restriction enzyme and cloned into the NcoI site of the above expression cassette containing plasmid, pUC18CP-exp. Clones containing the ZYMV coat protein gene insert need only be checked to determine correct orientation with respect with the CaMV promoter. However, to ensure that no artifacts have been incorporated during the PCR amplification the entire coat protein gene region is checked by nucleotide sequence analysis.

To obtain the amplified ZYMV coat protein gene with NcoI restriction enzyme sites on both ends the following two oligomers were synthesized:

1. 5'-ATCATTCCATGGGCACTCAACCAACTGTGGC-3', which adds a NcoI site to the 5'-end of the ZYMV coat protein gene and retains the same ATG translation start codon which is present in the expression cassette, pUC18cpexp.

2. 5'-AGCTAACCATGGCTAAAGATATCAAATAAAGCTG-3', which adds a NcoI site to the 3'-end of the ZYMV coat protein gene, this site can be ligated into the expression cassette, pUC18cpexp.

The cloning of this PCR ZYMV coat protein gene, using these two oligomers, into pUC18cpexp yields a plant expressible ZYMV gene (referred to as pUC18cpZYMV) which following transcription and translation will generate a ZYMV coat protein which is identical to that derived from the ZYMV coat protein gene nucleotide sequence, see Chart 3. However, this coat protein will differ, because of necessary genetic engineering to add the ATG initiation codon followed by Gly, which is the amino acid 3' adjacent to the Ser of the polyprot-

ein cleavage site (see Chart 3). The Gly amino acid residue was selected for the potential N-terminal amino acid because many potyvirus coat proteins have either an Ser, Gly, or Ala at their N-terminal. However, if this is found to be a problem its replacement would involve the use of a different oligomer to obtain a different N-terminal amino acid for the ZYMV coat protein. The cloned construction of the plant expressible ZYMV coat protein gene is referred to pUC18cpZYMV, and is shown in Chart 12.

Example 15 Construction of a Micro T-DNA Plasmid Containing the Plant-expressible ZYMV Coat Protein Gene Construction.

Following the teachings of Example 11 with appropriate modifications, the construction of a micro T-DNA plasmid containing a plant-expressible ZYMV coat protein was constructed. Plasmid pUC18cpZYMV (Chart 12) was digested with Hind III (which cuts within the polycloning sites of pUC18, well outside of the expression cassette), and a 1.6 kb fragment containing the plant-expressible cassette was removed and ligated into the Hind III site of the micro-vector pGA482 (Chart 7). The resulting plasmid was designated, pGA482GG/cpZYMV is shown in Chart 13.

After transfer of the engineered plasmid pGA482GG/cpZYMV into Agrobacterium strains, the Agrobacterium strains can be used to transfer and integrate within a plant genome the plant-expressible ZYMV coat protein gene contained within its T-DNA region.

Example 16 Microprojectile Transfer of pUC18cpZYMV into Plant Tissues.

Following the teachings of Example 12, the microprojectile transfer technique can be used to introduce the ZYMV coat protein gene with appropriate genetic regulatory sequences into plant tissues.

The use of this process for the transfer of pUC18cpZYMV can be accomplished after the addition of either plant expressible genes NPTII or Gus genes or both. Plasmids that have the nptII and Gus genes to pUC18cpZYMV are shown in Chart 14 and referred to as pUC18GcpZYMV and pUC18NGcpZYMV. In addition, the construction described in Example 15 can also be used for microprojectile transfer as it already has both the nptII and Gus genes attached to the pUC18cpZYMV cassette (see Chart 13). The only difficulty which the use of pGA482GG/cpZYMV may encounter during transfer by the

microprojectile process is due to its large size, about 18kb, which may have a lower efficiency transfer and such larger plasmid generally yield less DNA during propagation.

To construct plasmid pUC18GCPZYMV, plasmid pUC18CPZYMV is digested with BamHI and ligated to a 3.0 BamHI isolated fragment which contains the Gus gene. To construct plasmid pUC18GCPZYMV, plasmid pUC18GCPZYMV is digested with SmaI and ligated with a 2.4 kb isolated fragment containing the Nos nptII gene isolated by digestion with DraI and StuI.

CHARTS

CHART 1

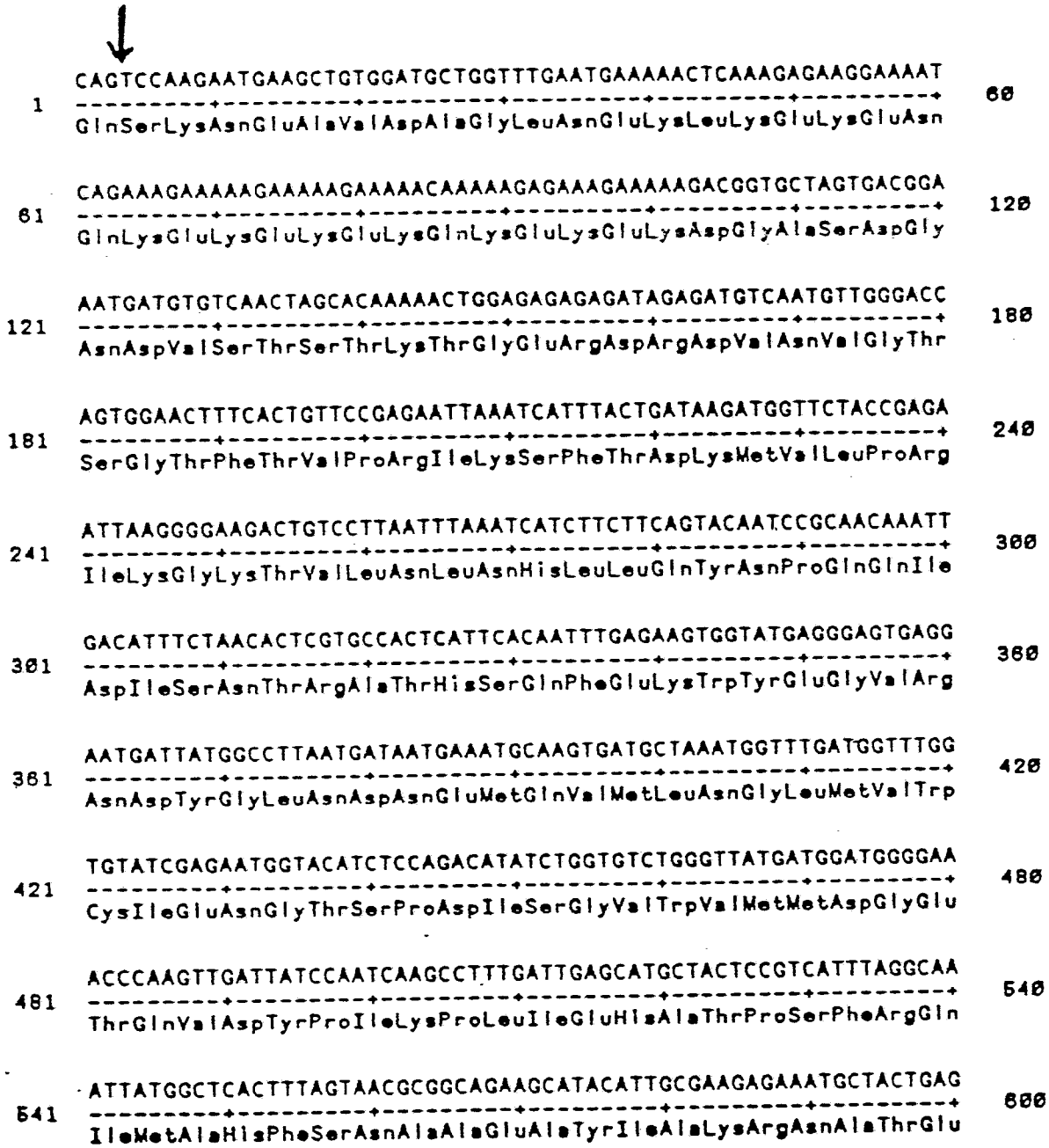


CHART 1 (Continued)

```
                AGGTACATGCCGCGGTATGGAATCAAGAGAAATTTGACTGACATTAGCCTCGCTAGATAC
      661       ---------+---------+---------+---------+---------+---------+      660
                ArgTyrMetProArgTyrGlyIleLysArgAsnLeuThrAspIleSerLeuAlaArgTyr

                GCTTTCGACTTCTATGAGGTGAATTCGAAAACACCTGATAGGGCTCGCGAAGCTCACATG
      661       ---------+---------+---------+---------+---------+---------+      720
                AlaPheAspPheTyrGluValAsnSerLysThrProAspArgAlaArgGluAlaHisMet

                CAGATGAAGGCTGCAGCGCTGCGAAACACCAGTCGCAAAATGTTTGGTATGGACGGCAGT
      721       ---------+---------+---------+---------+---------+---------+      780
                GlnMetLysAlaAlaAlaLeuArgAsnThrSerArgLysMetPheGlyMetAspGlySer

                GTTAGTAACAAGGAAGAAAACACGGAGAGACACACAGTGGAAGATGTCAATAGAGACATG
      781       ---------+---------+---------+---------+---------+---------+      840
                ValSerAsnLysGluGluAsnThrGluArgHisThrValGluAspValAsnArgAspMet

                CACTCTCTCCTGGGTATGCGCAACTAAATACCTGCGCTTGTGTGTTTGTTGAGTCTGACT
      841       ---------+---------+---------+---------+---------+---------+      900
                HisSerLeuLeuGlyMetArgAsnEnd

                CGACCCTGTTTCACCTTATGGTACTATATAAGCATTAGAATACAGAGTGGCTGCGCCACC
      901       ---------+---------+---------+---------+---------+---------+      960

                GCTTCTATTTTACAGTGAGGGTAGCCCTCCGTGCTTTTAGTATTATTCGAGTTCTCTGAG
      961       ---------+---------+---------+---------+---------+---------+     1020

                TCTCCATACAGTGTGGGTGGCCCACGTGATATTCGAGCCTCTTAGAATGAGAAAAAAAAA
     1021       ---------+---------+---------+---------+---------+---------+     1080

                AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGAATTCC
     1081       ---------+---------+---------+---------+----  1124
```

CHART 2

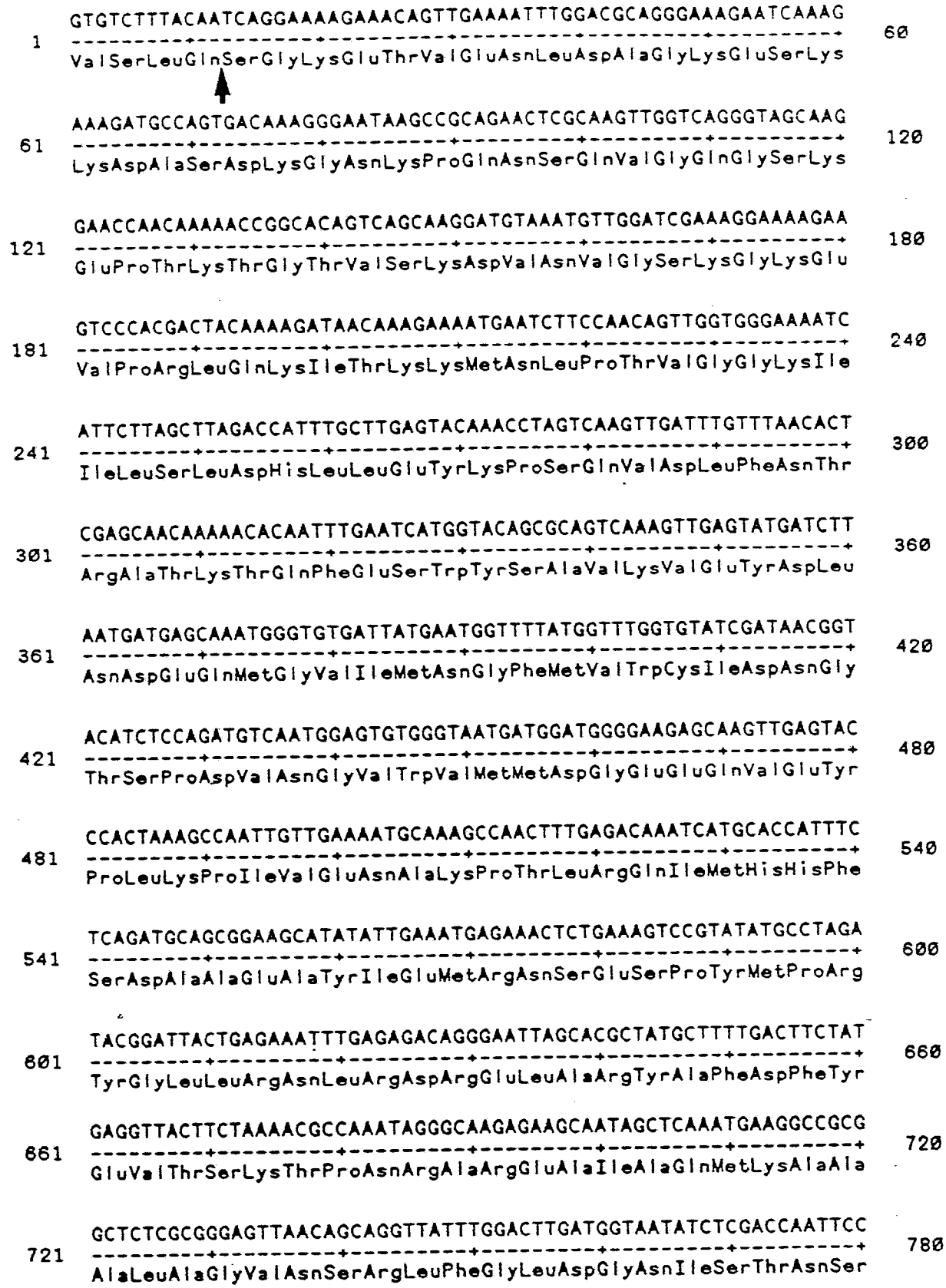

GTGTCTTTACAATCAGGAAAAGAAACAGTTGAAAATTTGGACGCAGGGAAAGAATCAAAG
1
60
ValSerLeuGlnSerGlyLysGluThrValGluAsnLeuAspAlaGlyLysGluSerLys
AAAGATGCCAGTGACAAAGGGAATAAGCCGCAGAACTCGCAAGTTGGTCAGGGTAGCAAG
61
120
LysAspAlaSerAspLysGlyAsnLysProGlnAsnSerGlnValGlyGlnGlySerLys
GAACCAACAAAAACCGGCACAGTCAGCAAGGATGTAAATGTTGGATCGAAAGGAAAAGAA
121
180
GluProThrLysThrGlyThrValSerLysAspValAsnValGlySerLysGlyLysGlu
GTCCCACGACTACAAAAGATAACAAAGAAAATGAATCTTCCAACAGTTGGTGGGAAAATC
181
240
ValProArgLeuGlnLysIleThrLysLysMetAsnLeuProThrValGlyGlyLysIle
ATTCTTAGCTTAGACCATTTGCTTGAGTACAAACCTAGTCAAGTTGATTTGTTTAACACT
241
300
IleLeuSerLeuAspHisLeuLeuGluTyrLysProSerGlnValAspLeuPheAsnThr
CGAGCAACAAAAACACAATTTGAATCATGGTACAGCGCAGTCAAAGTTGAGTATGATCTT
301
360
ArgAlaThrLysThrGlnPheGluSerTrpTyrSerAlaValLysValGluTyrAspLeu
AATGATGAGCAAATGGGTGTGATTATGAATGGTTTTATGGTTTGGTGTATCGATAACGGT
361
420
AsnAspGluGlnMetGlyValIleMetAsnGlyPheMetValTrpCysIleAspAsnGly
ACATCTCCAGATGTCAATGGAGTGTGGGTAATGATGGATGGGGAAGAGCAAGTTGAGTAC
421
480
ThrSerProAspValAsnGlyValTrpValMetMetAspGlyGluGluGlnValGluTyr
CCACTAAAGCCAATTGTTGAAAATGCAAAGCCAACTTTGAGACAAATCATGCACCATTTC
481
540
ProLeuLysProIleValGluAsnAlaLysProThrLeuArgGlnIleMetHisHisPhe
TCAGATGCAGCGGAAGCATATATTGAAATGAGAAACTCTGAAAGTCCGTATATGCCTAGA
541
600
SerAspAlaAlaGluAlaTyrIleGluMetArgAsnSerGluSerProTyrMetProArg
TACGGATTACTGAGAAATTTGAGAGACAGGGAATTAGCACGCTATGCTTTTGACTTCTAT
601
660
TyrGlyLeuLeuArgAsnLeuArgAspArgGluLeuAlaArgTyrAlaPheAspPheTyr
GAGGTTACTTCTAAAACGCCAAATAGGGCAAGAGAAGCAATAGCTCAAATGAAGGCCGCG
661
720
GluValThrSerLysThrProAsnArgAlaArgGluAlaIleAlaGlnMetLysAlaAla
GCTCTCGCGGGAGTTAACAGCAGGTTATTTGGACTTGATGGTAATATCTCGACCAATTCC
721
780
AlaLeuAlaGlyValAsnSerArgLeuPheGlyLeuAspGlyAsnIleSerThrAsnSer

CHART 2 (Continued)

```
                GAAAATACTGGGAGGCACACTGCAAGGGACGTAAATCAGAATATGCATACTTTGTTGGGT
781             ---------+---------+---------+---------+---------+---------+  840
                GluAsnThrGlyArgHisThrAlaArgAspValAsnGlnAsnMetHisThrLeuLeuGly

                ATGGGTCCACCGCAGTAAAGACTAGGTAAACTGGTCACAGTTAGCATTTCGGGTCGTTAT
841             ---------+---------+---------+---------+---------+---------+  900
                MetGlyProProGlnEnd

                AAGTTTTCTATAATATAACATGTCGCACTTTATTTTAGTATAGTGTGATTTCATCACGTT
901             ---------+---------+---------+---------+---------+---------+  960

                TGTACTGTTTATGTTAGCGTGGTTTAACCACCTTTGTGTGTGCTTTATATTATAGTTTAT
961             ---------+---------+---------+---------+---------+---------+  1020

                GCGTAGCAGGGAGAACCATTACAATGCCGGAGTTGTTTGTAGTGTGATTTCATCACGGTT
1021            ---------+---------+---------+---------+---------+---------+  1080

                AATAGCCGAGGTACGGTAATGTTTGTTGCCTAAAAAAAAAAAAAAAAAAAAAAAA
1081            ---------+---------+---------+---------+---------+-----  1135
```

CHART 3

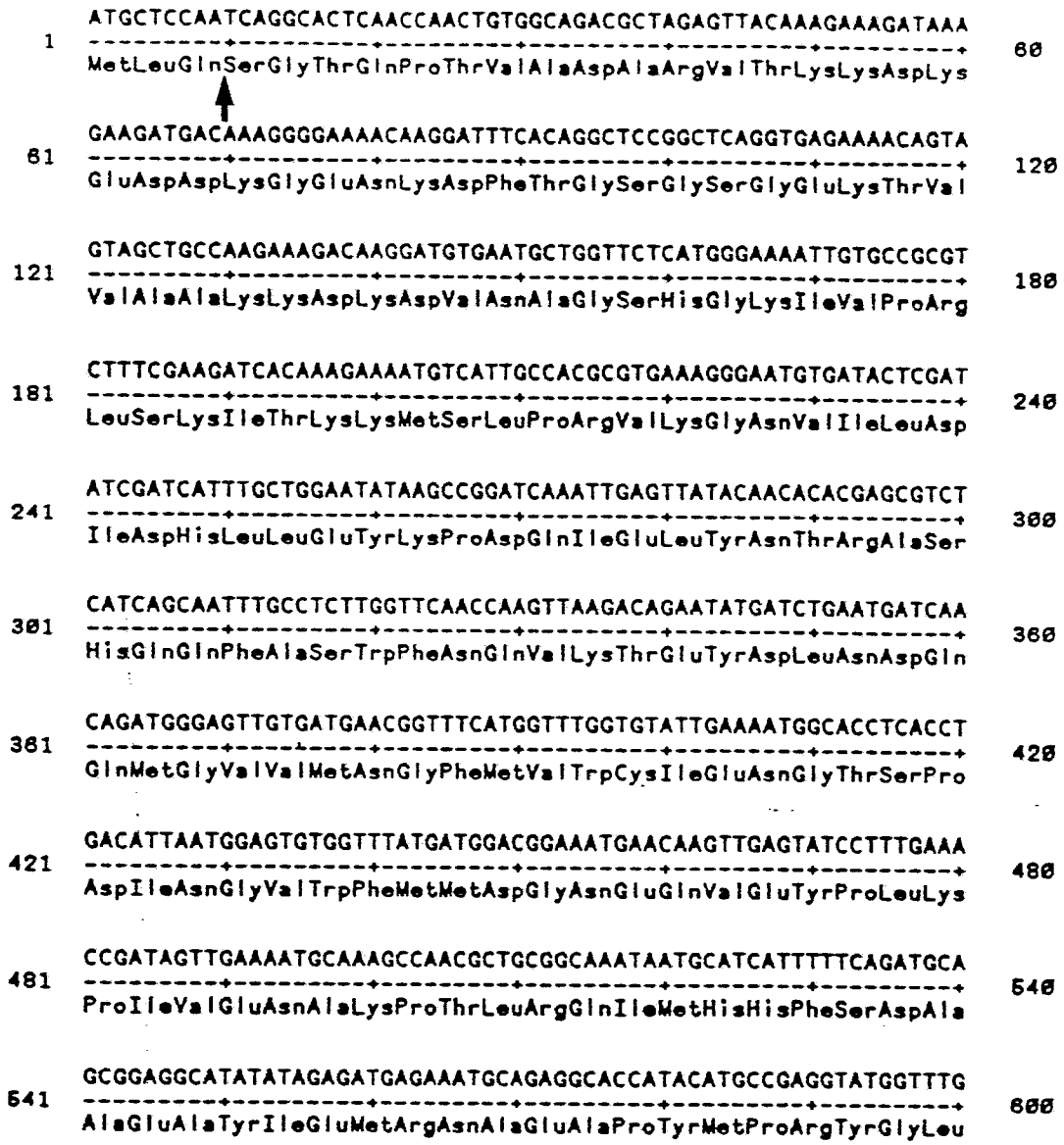

ATGCTCCAATCAGGCACTCAACCAACTGTGGCAGACGCTAGAGTTACAAAGAAAGATAAA
MetLeuGlnSerGlyThrGlnProThrValAlaAspAlaArgValThrLysLysAspLys
GAAGATGACAAAGGGGAAAACAAGGATTTCACAGGCTCCGGCTCAGGTGAGAAAACAGTA
GluAspAspLysGlyGluAsnLysAspPheThrGlySerGlySerGlyGluLysThrVal
GTAGCTGCCAAGAAAGACAAGGATGTGAATGCTGGTTCTCATGGGAAAATTGTGCCGCGT
ValAlaAlaLysLysAspLysAspValAsnAlaGlySerHisGlyLysIleValProArg
CTTTCGAAGATCACAAAGAAAATGTCATTGCCACGCGTGAAAGGGAATGTGATACTCGAT
LeuSerLysIleThrLysLysMetSerLeuProArgValLysGlyAsnValIleLeuAsp
ATCGATCATTTGCTGGAATATAAGCCGGATCAAATTGAGTTATACAACACACGAGCGTCT
IleAspHisLeuLeuGluTyrLysProAspGlnIleGluLeuTyrAsnThrArgAlaSer
CATCAGCAATTTGCCTCTTGGTTCAACCAAGTTAAGACAGAATATGATCTGAATGATCAA
HisGlnGlnPheAlaSerTrpPheAsnGlnValLysThrGluTyrAspLeuAsnAspGln
CAGATGGGAGTTGTGATGAACGGTTTCATGGTTTGGTGTATTGAAAATGGCACCTCACCT
GlnMetGlyValValMetAsnGlyPheMetValTrpCysIleGluAsnGlyThrSerPro
GACATTAATGGAGTGTGGTTTATGATGGACGGAAATGAACAAGTTGAGTATCCTTTGAAA
AspIleAsnGlyValTrpPheMetMetAspGlyAsnGluGlnValGluTyrProLeuLys
CCGATAGTTGAAAATGCAAAGCCAACGCTGCGGCAAATAATGCATCATTTTTCAGATGCA
ProIleValGluAsnAlaLysProThrLeuArgGlnIleMetHisHisPheSerAspAla
GCGGAGGCATATATAGAGATGAGAAATGCAGAGGCACCATACATGCCGAGGTATGGTTTG
AlaGluAlaTyrIleGluMetArgAsnAlaGluAlaProTyrMetProArgTyrGlyLeu
1
60
61
120
121
180
181
240
241
300
301
360
361
420
421
480
481
540
541
600

CHART 3 (Continued)

```
      CTTCGAAACCTACGGGATAGGAGTTTAGCACGATACGCTTTCGATTTCTATGAAGTCAAT
601   ---------+---------+---------+---------+---------+---------+  660
      LeuArgAsnLeuArgAspArgSerLeuAlaArgTyrAlaPheAspPheTyrGluValAsn

      TCTAAAACTCCTGAAAGAGCCCATGAAGCTGTTGCGCAGATGAAAGCAGCAGCTCTTAGC
661   ---------+---------+---------+---------+---------+---------+  720
      SerLysThrProGluArgAlaHisGluAlaValAlaGlnMetLysAlaAlaAlaLeuSer

      AATGTTTCTTCAAGTGTGTTTGGCCTTAGTGAAATCGTTGCCACCACTAGCGAAGCCACA
721   ---------+---------+---------+---------+---------+---------+  780
      AsnValSerSerSerValPheGlyLeuSerGluIleValAlaThrThrSerGluAlaThr

      CTGAACGGCACACTGCACGTGATGTTAATAGAAACATGCCACACCTTACTAGGTGTGAAT
781   ---------+---------+---------+---------+---------+---------+  840
      LeuAsnGlyThrLeuHisValMetLeuIleGluThrCysHisThrLeuLeuGlyValAsn

      ACAATGCAGTAAAGGGTAGGCCGCCTACCTAGGTTATCGCTTCGCTGCCGACGTAATTCT
841   ---------+---------+---------+---------+---------+---------+  900
      ThrMetGlnEnd

      AATATTTACCAGCTTTATTTGATATCTTTAGATTTCCAGAGTGGGCCTCCCACCTTTAAA
901   ---------+---------+---------+---------+---------+---------+  960

      GCGTAGAGTTTATGCTTAGTTGTCCAGGAGTGCCGTAGTCCTGTCGGAAGCTTTAGTGTG
961   ---------+---------+---------+---------+---------+---------+ 1020

      AGCCTCTCACGAATAAGCTCGAGATTAGACTCCGTTTGCAAGCCTAAAAAAAAAAAAAAA
1021  ---------+---------+---------+---------+---------+---------+ 1080

      AAAA
1081  ----  1084
```

CHART 4

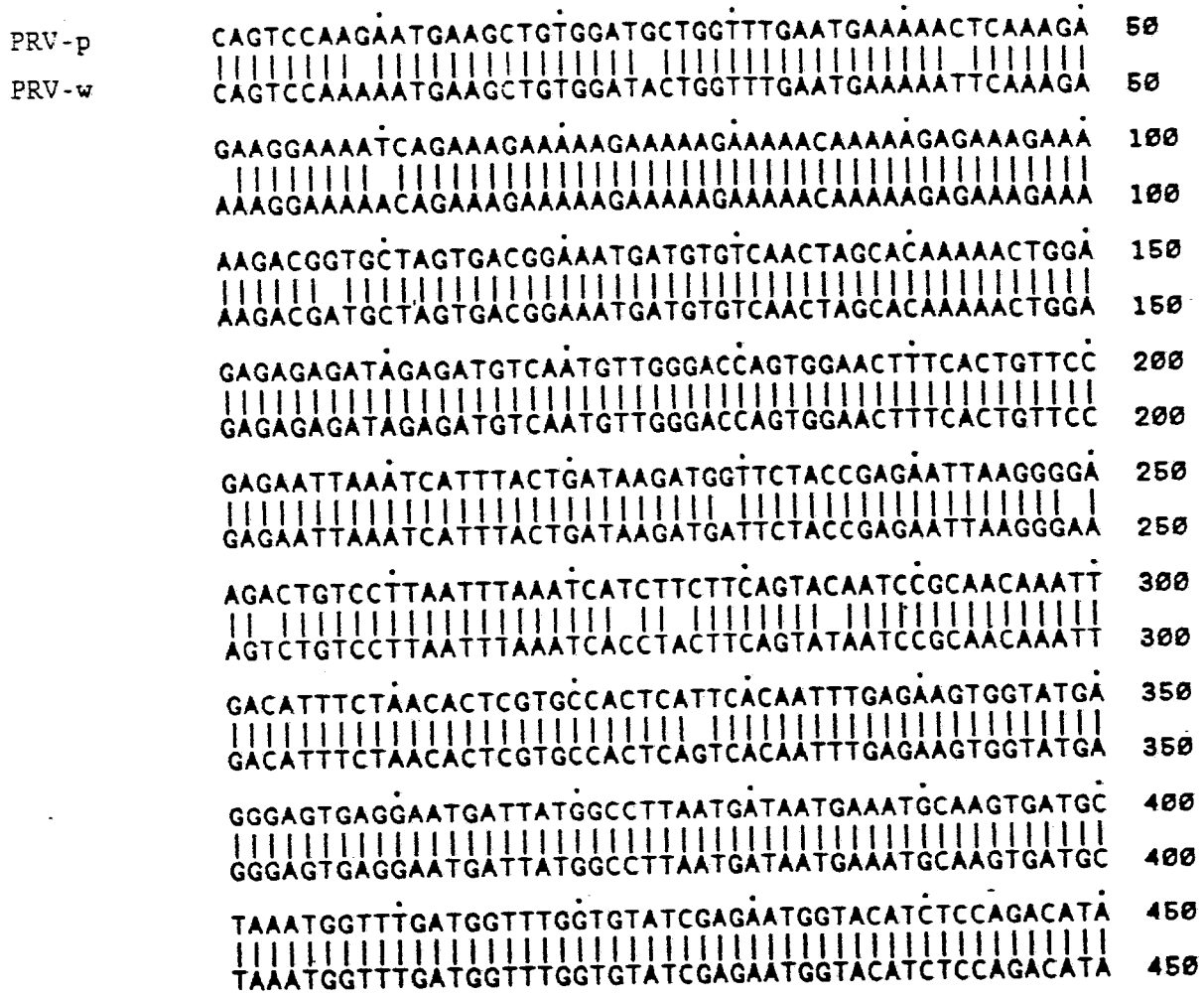

PRV-p
PRV-w
CAGTCCAAGAATGAAGCTGTGGATGCTGGTTTGAATGAAAAACTCAAAGA 50
CAGTCCAAAAATGAAGCTGTGGATACTGGTTTGAATGAAAAATTCAAAGA 50
GAAGGAAAATCAGAAAGAAAAGAAAAAGAAAAACAAAAAGAGAAAGAAA 100
AAAGGAAAAACAGAAAGAAAAAGAAAAAGAAAAACAAAAAGAGAAAGAAA 100
AAGACGGTGCTAGTGACGGAAATGATGTGTCAACTAGCACAAAAACTGGA 150
AAGACGATGCTAGTGACGGAAATGATGTGTCAACTAGCACAAAAACTGGA 150
GAGAGAGATAGAGATGTCAATGTTGGGACCAGTGGAACTTTCACTGTTCC 200
GAGAGAGATAGAGATGTCAATGTTGGGACCAGTGGAACTTTCACTGTTCC 200
GAGAATTAAATCATTTACTGATAAGATGGTTCTACCGAGAATTAAGGGGA 250
GAGAATTAAATCATTTACTGATAAGATGATTCTACCGAGAATTAAGGGAA 250
AGACTGTCCTTAATTTAAATCATCTTCTTCAGTACAATCCGCAACAAATT 300
AGTCTGTCCTTAATTTAAATCACCTACTTCAGTATAATCCGCAACAAATT 300
GACATTTCTAACACTCGTGCCACTCATTCACAATTTGAGAAGTGGTATGA 350
GACATTTCTAACACTCGTGCCACTCAGTCACAATTTGAGAAGTGGTATGA 350
GGGAGTGAGGAATGATTATGGCCTTAATGATAATGAAATGCAAGTGATGC 400
GGGAGTGAGGAATGATTATGGCCTTAATGATAATGAAATGCAAGTGATGC 400
TAAATGGTTTGATGGTTTGGTGTATCGAGAATGGTACATCTCCAGACATA 450
TAAATGGTTTGATGGTTTGGTGTATCGAGAATGGTACATCTCCAGACATA 450

CHART 4 (Continued)

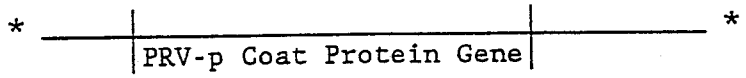
```
TCTGGTGTCTGGGTTATGATGGATGGGGAAACCCAAGTTGATTATCCAAT  500
||||||||||||||||||||||||||||||||||||||||||||||||||
TCTGGTGTCTGGGTTATGATGGATGGGGAAACCCAAGTTGATTATCCAAT  500

CAAGCCTTTGATTGAGCATGCTACTCCGTCATTTAGGCAAATTATGGCTC  550
|||||||||| ||||||||||||||||||||||||||||||||||||||
CAAGCCTTTAATTGAGCATGCTACTCCGTCATTTAGGCAAATTATGGCTC  550

ACTTTAGTAACGCGGCAGAAGCATACATTGCGAAGAGAAATGCTACTGAG  600
|||||||||||||||||||||||||||||||||||| ||||||||||||||
ACTTTAGTAACGCGGCAGAAGCATACATTGCGAAAAGAAATGCTACTGAG  600

AGGTACATGCCGCGGTATGGAATCAAGAGAAATTTGACTGACATTAGCCT  650
||||||||||||||||||||||||||||||||||||||||||||||||||
AGGTACATGCCGCGGTATGGAATCAAGAGAAATTTGACTGACATTAGCCT  650

CGCTAGATACGCTTTCGACTTCTATGAGGTGAATTCGAAAACACCTGATA  700
||||||||||||||||||||||||||||||||||||||||||||||||||
CGCTAGATACGCTTTCGACTTCTATGAGGTGAATTCGAAAACACCTGATA  700

GGGCTCGCGAAGCTCACATGCAGATGAAGGCTGCAGCGCTGCGAAACACC  750
|||||||||||||| |||||||||||||||||||||| ||||||||||||
GGGCTCGCGAAGCCCACATGCAGATGAAGGCTGCAGCACTGCGAAACACT  750

AGTCGCAAAATGTTTGGTATGGACGGCAGTGTTAGTAACAAGGAAGAAAA  800
||||||| ||||||||||||||||||||||||||||||||||||||||||
AGTCGCAGAATGTTTGGTATGGACGGCAGTGTTAGTAACAAGGAAGAAAA  800

CACGGAGAGACACACAGTGGAAGATGTCAATAGAGACATGCACTCTCTCC  850
|||||||||||||||||||||||| ||||||||||||||||||||||||
CACGGAGAGACACACAGTGGAAGACGTCAATAGAGACATGCACTCTCTCC  850

TGGGTATGCGCAACTAA  867
||||||||||||||||
TGGGTATGCGCAACTAA  867
```

CHART 5

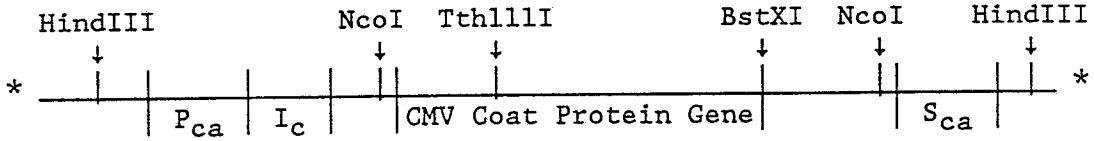
```
SMV    SGKEKEGDMDADKDPKKSTSSSKG.................AGTSSKDVNV 34
       ||||  ||||||||  ||  |                    ||  ||||||
WMVII  SGKETVENLDAGKESKKDASDKGNKPQNSQVGQGSKEPTKTGTVSKDVNV 50

SMV    GSKGKVVPRLQKITRKMNLPMVEGKIILSLDHLLEYKPNQVDLFNTRATR 84
       |||||  ||||||||||||||  ||||||||||||||||||  ||||||||||
WMVII  GSKGKEVPRLQKITKKMNLPTVGGKIILSLDHLLEYKPSQVDLFNTRATK 100

SMV    TQFEAWYNAVKDEYELDDEQMGVVMNGFMVWCIDNGTSPDANGVWVMMDG 134
       ||||  ||  |||  ||||||||||||||||||||||||||||  |||||||||
WMVII  TQFESWYSAVKVEYDLNDEQMGVIMNGFMVWCIDNGTSPDVNGVWVMMDG 150

SMV    EEQIEYPLKPIVENAKPTLRQIMHHFSDAAEAYIEMRNSESPYMPRYGLL 184
       ||||||||||||||||||||||||||||||||||||||||||||||||||||
WMVII  EEQVEYPLKPIVENAKPTLRQIMHHFSDAAEAYIEMRNSESPYMPRYGLL 200

SMV    RNLRDRELARYAFDFYEVTSKTPNRAREAIAQMKAAALSGVNNKLFGLDG 234
       ||||||||||||||||||||||||||||||||||||  |||  |||||||
WMVII  RNLRDRELARYAFDFYEVTSKTPNRAREAIAQMKAAALAGVNSRLFGLDG 250

SMV    NISTNSENTERHTARDVNQNMHTLLGMGPPQ 265
       ||||||||||||||||||||||||||||||
WMVII  NISTNSENTGRHTARDVNQNMHTLLGMGPPQ 281
```

CHART 6

pPRV117



pUC1813/Cp19



pUC1813/Cp19-PRVexp

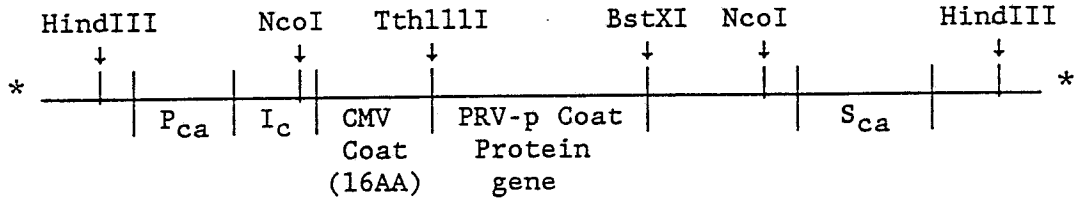
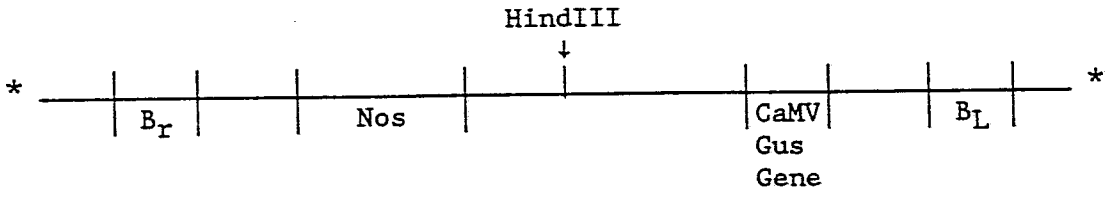


CHART 7

pGA482



pGA482/G/CP19-PRVexp

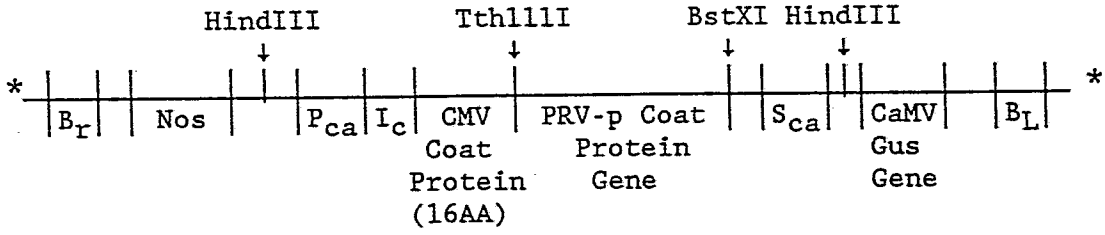
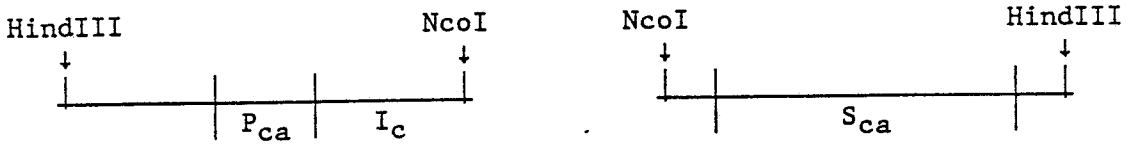


CHART 8



p18CaMV/CMV-exp

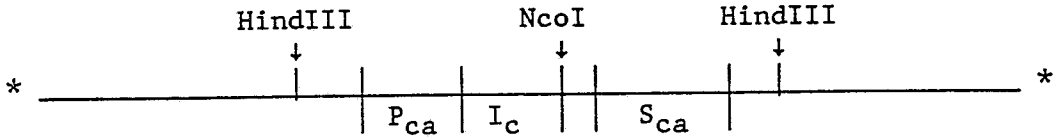
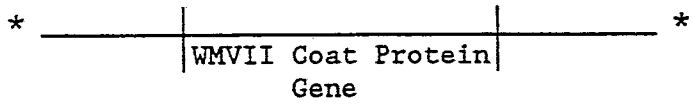
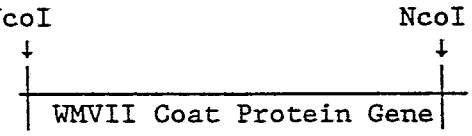


CHART 9

pWMVII-41-3.2



PCR Generated Gene



p18WMVII-exp

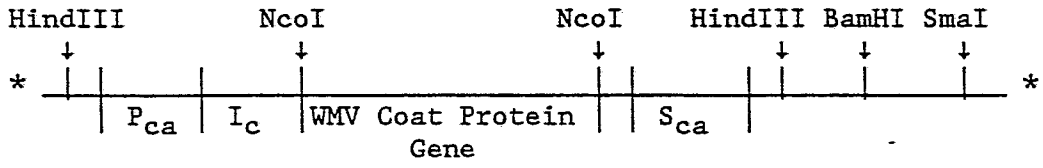


CHART 10

pGA482/G/CPWMVII-exp

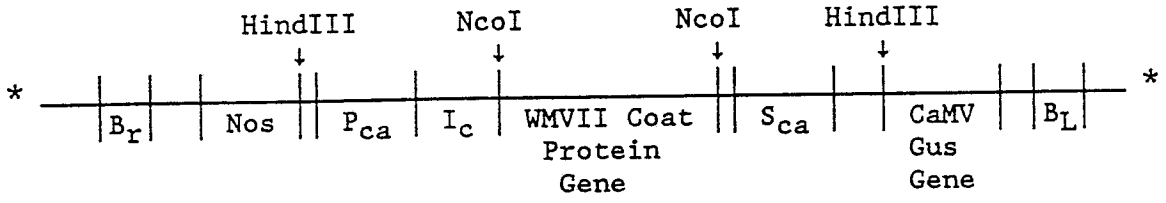
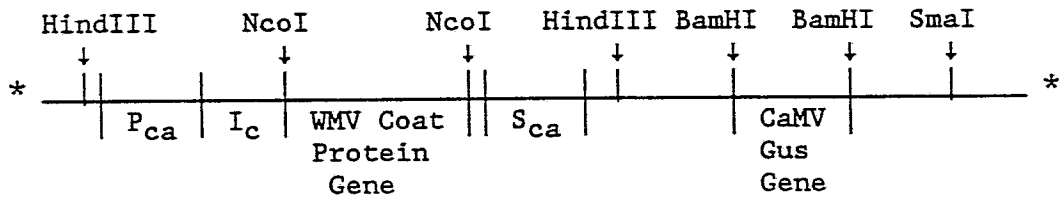


CHART 11

p18GWMVII-exp



p18NGWMVII-exp

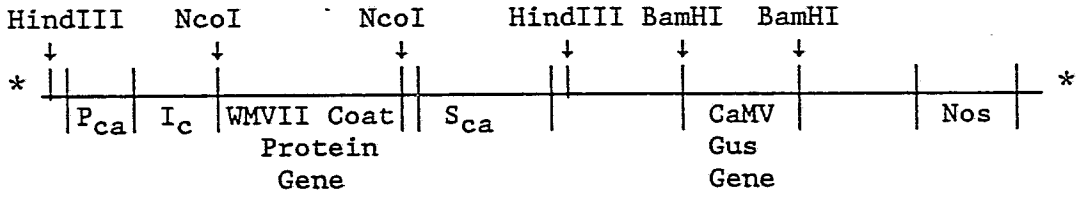
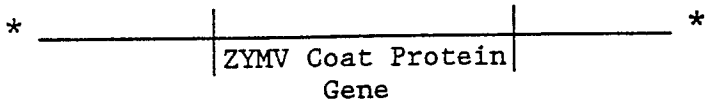
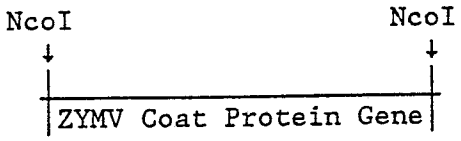


CHART 12

pZYMV-15



ZYMV Coat Protein Gene



pUC18CpZYMV

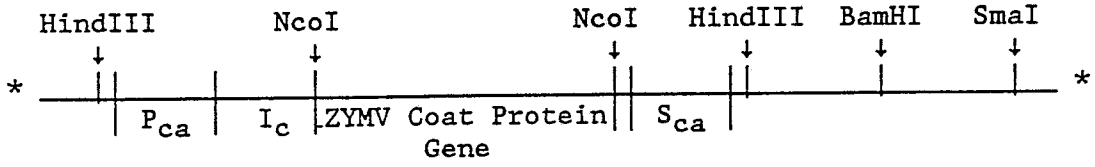


CHART 13

pGA482/GG/cpZYMV

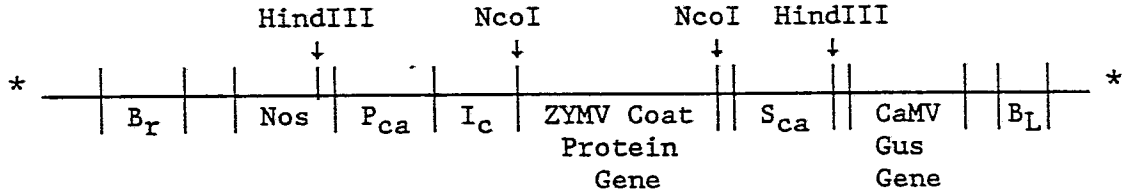
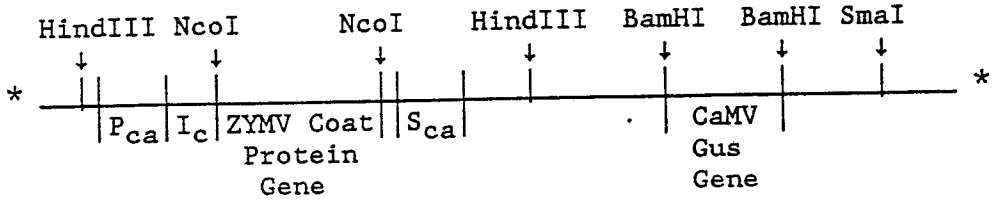
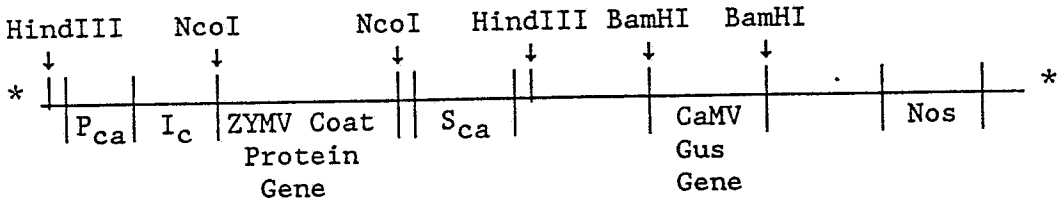


CHART 14

pUC18GCpZYMV



pUC18NGCpZYMV



CLAIMS

1. A recombinant DNA molecule which encodes a potyvirus coat protein, said recombinant DNA molecule selected from the group consisting of Papaya ringspot virus strain papaya ringspot PRV-p coat protein gene, Watermelon mosaic virus II WMVII coat protein gene, and Zucchini yellow mosaic virus ZYMV coat protein gene.

2. A recombinant DNA molecule according to Claim 1 wherein said recombinant DNA molecule encodes Papaya ringspot virus strain papaya ringspot PRV-p coat protein, said recombinant DNA molecule having the nucleotide sequence shown in Chart 1.

3. A recombinant DNA molecule according to Claim 1 wherein said recombinant DNA molecule encodes Watermelon mosaic virus II WMVII coat protein, said recombinant DNA molecule having the nucleotide sequence shown in Chart 2.

4. A recombinant DNA molecule according to Claim 1 wherein said recombinant DNA molecule encodes Zucchini yellow mosaic virus ZYMV coat protein, said recombinant DNA molecule having the nucleotide sequence shown in Chart 3.

5. A recombinant DNA molecule according to Claim 1 further comprising:

a) a promoter;

b) an initiation region; and,

c) a poly(A) addition signal;

wherein said promoter is upstream and operably linked to said initiation region, said initiation region is upstream and operably linked to said recombinant DNA molecule encoding a coat protein, and said recombinant DNA molecule encoding a coat protein is upstream and operably linked to said poly(A) addition signal.

6. A recombinant DNA molecule according to Claim 5 wherein said promoter is Cauliflower mosaic virus CaMV 35S promoter.

7. A recombinant DNA molecule according to Claim 5 wherein said initiation region is selected from the group consisting of an

initiation region derived from the 5' untranslated region of Cucumber mosaic virus CMV coat protein gene and an initiation region derived from the 5' untranslated region of SS RUBISCO gene.

8. A recombinant DNA molecule according to Claim 5 wherein said initiation region comprises the sequence AAXXATGG.

9. A recombinant DNA molecule according to Claim 5 wherein said poly(A) addition signal is selected from the group consisting of: the poly(A) signal derived from Cauliflower mosaic virus CaMV 35S gene; the poly(A) signal derived from phaseolin storage protein gene; the poly(A) signal derived from nopaline synthase gene; the poly(A) signal derived from octopine synthase gene; the poly(A) signal derived from bean storage protein gene; and, the poly(A) signal derived from SS RUBISCO.

10. A recombinant DNA molecule according to Claim 6 wherein said initiation region is derived from the 5' untranslated region of Cucumber mosaic virus CMV coat protein gene and said poly(A) addition signal is derived from Cauliflower mosaic virus CaMV 35S gene.

11. A recombinant DNA molecule according to Claim 5 further comprising an AT rich 5' untranslated region wherein:

a) said AT rich region is downstream from said promoter and upstream from said initiation region;

b) said initiation region comprises the sequence AAXXATGG; and,

c) said poly(A) addition signal contains untranslated flanking sequences.

12. A recombinant DNA molecule according to Claim 14 wherein said promoter is Cauliflower mosaic virus CaMV 35S promoter.

13. A recombinant DNA molecule according to Claim 11 wherein said AT-rich 5' untranslated region is derived from the 5' untranslated region of a gene selected from the group consisting of Cucumber mosaic virus CMV coat protein gene and SS RUBISCO gene.

14. A recombinant DNA molecule according to Claim 11 wherein said initiation region is derived from the 5' untranslated region of a gene selected from the group consisting of Cucumber mosaic virus CMV coat protein gene and SS RUBISCO gene.

15. A recombinant DNA molecule according to Claim 11 wherein said initiation region comprises the sequence AAXXATGG.

16. A recombinant DNA molecule according to Claim 11 wherein said poly(A) addition signal is selected from the group consisting of: the poly(A) signal derived from Cauliflower mosaic virus CaMV 35S gene; the poly(A) signal derived from phaseolin storage protein gene; the poly(A) signal derived from nopalinesynthase gene; the poly(A) signal derived from octopine synthase gene; the poly(A) signal derived from bean storage protein gene; and, the poly(A) signal derived from SS RUBISCO.

17. A recombinant DNA molecule according to Claim 12 wherein;

a) said AT rich 5' untranslated region and said initiation region are derived from the 5' untranslated region of Cucumber mosaic virus CMV coat protein gene;

b) said initiation region comprises the sequence AAXXATGG; and,

c) and said poly(A) addition signal is derived from Cauliflower mosaic virus CaMV 35S gene.

18. A transformed plant cell containing a recombinant DNA molecule according to Claim 5.

19. A transformed plant cell according to Claim 18 containing a recombinant DNA molecule according to Claim 10.

20. A transformed plant cell according to Claim 18 containing a recombinant DNA molecule according to Claim 11.

21. A transformed plant cell according to Claim 20 containing a recombinant DNA molecule according to Claim 17.

22. A transgenic plant selected from the group consisting of the families Cucuribitaceae, Caricaceae, Solanaceae, and Leguminoseae comprising transformed plant cells according to Claim 18.

23. A transgenic plant according to Claim 22 comprising transformed plant cells according to Claim 19.

24. A transgenic plant according to Claim 22 comprising transformed plant cells according to Claim 20.

25. A transgenic plant according to Claim 24 comprising transformed plant cells according to Claim 21.

26. A process for producing a transgenic plant which is resistant to viral infection comprising the steps of:

a) constructing a recombinant DNA molecule according to Claim 5;

b) transforming plant cells with said recombinant DNA; and

c) regenerating plants from said transformed plant cells.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/03094

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 12 N 15/40, C 12 N 15/82, C 12 N 5/14, A 01 H 4/00, A 01 N 63/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 12 N, A 01 H, A 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
O,X	Phytopatology, vol. 77, no. 1, 1987, B. L'Hostis et al.: "Synthesis and cloning of a DNA complementary to a mild mutant of papaya ringspot virus", page 119, & Annual Meeting of the American Phytopathological Society (Northeastern Division), 5-7 November 1986, Phytopathology 77 (1) 1987, 119, see the abstract	1,2
O,Y		5,6,9,18,22, 26
	--	
O,X	Hortscience, vol. 23, no. 3, June 1988, R. Grumet et al.: "Purification and cloning of Zucchini yellows mosaic virus", page 755, abstract no. 260, & 85th Annual Meeting of the American Society for Horticultural Science and the 33rd Annual Meeting of the Canadian Society for Horticultural Science, East Lansing, Michigan, USA, 6-12 August 1988 Hortscience 23 (3 sect. 2), 1988, 755	1,4

./.

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10th October 1989	06. 12. 89
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	F.M. VRIJDAG

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	see the abstract	
O,Y	--	5,6,9,18, 22,26
Y	EP, A, 0223452 (MONSANTO) 27 May 1987, see page 5, lines 6-10; example 1	5,6,9,18, 22,26
	--	
A	Biological Abstracts, vol. 88, 1989, M.H. Yu et al.: "Cost protein of potyviruses: 6. Amino acid sequences suggest watermelon mosaic virus 2 and soybean mosaic virus N are strains of the same potyvirus", abstract no. 44985, & Arch. Virol. 105(1/2): 55-64, 1989, see the abstract	1,3
	--	
O,A	Journal of Cellular Biochemstry, UCLA Symposia on Molecular & Cellular Biology, Abstracts of the 17th Annual Meetings, 28 February - 10 April 1988, Supplement 12C, 1988, Alan R. Liss, Inc. (New York, US), A.L. Eggenberger et al.: "cDNA cloning, sequencing, and expression of the soybean mosaic virus coat protein coding sequence", page 274, abstract no. Y 240, see the abstract	1,3

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8903094 SA 30293

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 22/11/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0223452	27-05-87	AU-A- 6452886	30-04-87
		JP-A- 62201527	05-09-87